Journal of Forensic and Legal Medicine 20 (2013) 785–787

Contents lists available at SciVerse ScienceDirect

Journal of Forensic and Legal Medicine

journal homepage: www.elsevier.com/locate/jflm

Original communication

Why are Wischnewski spots not always present in lethal hypothermia? The results of testing a stress-reduced animal model

Fiona Bright, BHthSci (Hons) PhD Student, Calle Winskog, MD Forensic Pathologist, Melissa Walker, BSc Research Assistant, Roger W. Byard, MD Forensic Pathologist, Prof. *

Discipline of Anatomy and Pathology, The University of Adelaide, Frome Road, Adelaide, SA 5005, Australia

ARTICLE INFO

Article history:
Received 18 March 2013
Accepted 31 May 2013
Available online 25 June 2013

Keywords:
Hypothermia
Stress
Wischnewski spots
Animal model

ABSTRACT

Hypothermic fatalities in humans are characterized by a range of often subtle pathological findings that typically include superficial erosive gastritis (Wischnewski spots). Experimental studies have been successfully performed using animal models to replicate this finding, however study animals have inevitably been subjected to a variety of additional stressors including food deprivation, restraint and partial immersion in water while conscious. As it is recognised that stress on its own may cause superficial erosive gastritis, a model has been developed to enable the study of the effects of hypothermia in isolation. 42 Sprague–Dawley rats were allowed free social contact and were fed and watered ad libitum prior to being anaesthetized with isoflurane. Once unconscious, rats were placed on drape cloth covering metal mesh platforms in a styrofoam box packed with ice. The apparatus enabled both maintenance of a specific low temperature (26 °C) in 14 animals, and continued reduction of core temperatures in the remaining 28 (who all died of hypothermia under anaesthesia). Examination of the gastric mucosa in both groups macroscopically and microscopically failed to demonstrate typical Wischnewski spots in any of the 42 animals. Thus, in this model, death from hypothermia occurred without the development of these lesions. These results suggest that stress may be a significant effect modifier in the development of Wischnewski spots in lethal hypothermia.

1. Introduction

Hypothermia in humans refers to a situation where the core temperature has fallen to less than 35 °C. The causes of such a reduction in body temperature are quite diverse and involve both external factors, such as exposure to low environmental temperatures, and internal factors, related to diseases that interfere with normal thermoregulation.[1–5] Death may occur when the core temperature falls below 26–29 °C, and usually results from cardiac arrhythmias.[2,3]

The diagnosis of hypothermia may be difficult, as core and environmental temperatures at the death scene are usually not available, unless a pathologist has attended.[6] Thus, subtle (and nonspecific) markers at autopsy such as pink discolouration of the skin, pancreatitis, and pulmonary oedema are often sought. Although not considered pathognomonic of hypothermia, Wischnewski spots in the gastric mucosa are regarded as the most reliable indicator of significantly reduced core temperatures.[2–4,7] However, the underlying mechanisms for the development of these pathological changes remain poorly understood[1] and their pathogenesis has been attributed to disturbances in tissue microcirculation with ischaemic-reperfusion injury, the actions of tissue amines, and the breakdown of haemoglobin.[3,8–11]

An additional factor that has been proposed in the pathogenesis of Wischnewski spots is stress[7,9,12] and animal models using rats have been developed to help assess this association. Landeira-Fernandez found that rats that were conscious and exposed to cold water stress developed the most severe gastric lesions. This led the authors to suggest that it is the integrity of the higher order processing systems that plays an important role in the development of gastric ulceration during cold exposure.[13] Vincent et al. reported that decreasing stress by providing access to a biting mechanism during cold exposure, counteracted the effects of hypothermia, with rats showing fewer and smaller gastric lesions.[14] Goldman and Rossof were able to produce gastric mucosal ulcers in an experiment that did not expose rats to cold temperatures, but instead stressed them by fasting and immobilizing them in wire mesh for up to 20 h[15] While these studies suggest that stress may be a significant factor in the development of superficial gastric

* Corresponding author. School of Health Sciences, Level 3 Medical School North Building, The University of Adelaide, Frome Road, Adelaide, SA 5005, Australia. Tel.: +61 (618) 8303 5441; fax: +61 (618) 8303 4408.
E-mail address: roger.byard@sa.gov.au (R.W. Byard).

1752-928X/$ – see front matter © 2013 Elsevier Ltd and Faculty of Forensic and Legal Medicine. All rights reserved.
http://dx.doi.org/10.1016/j.jflm.2013.05.003

erosions, it remains unclear whether Wischnewski spots may be caused by stress alone, a combination of stress and hypothermia, or from pure hypothermia.

As previous studies have stressed rats by depriving them of food, exposing them to cold water or immobilizing them,[1] it was decided to develop a stress-reduced animal model that could be used to more carefully evaluate the pathological effects on the gastric mucosa of pure hypothermia.

2. Materials and methods

42 male Sprague–Dawley rats (21 young – 3 months, and 21 aged – 8–24 months) were used. The two age groups were chosen in case age had a significant effect on the pathological outcome. Males were chosen as they are more vulnerable to the effects of cold than females.[16,17] The rats were allowed unrestricted social contact and were fed and watered ad libitum prior to the study. The rats were handled infrequently and gently, and anaesthetized in an induction chamber using a Stinger® machine with an oxygen flow rate of 1 L per minute and 4–5% isoflurane. The latter rate was decreased to approximately 2–3%, and then to 1% during the procedure to sustain deep sleep while not significantly depressing respiration. Once unconscious, the rats were placed on a drape cloth which covered metal mesh platforms in a styrofoam box packed with ice. The animals were surrounded by, and in contact with, ice but were not covered, to enable visual monitoring of their breathing and heart rate (Fig. 1). The state of consciousness was monitored by assessing foot reflex for the entire procedure. A rectal temperature probe (Maverick® ET-7 wireless thermometer) was inserted 3 cm into the rectum of each rat. Core body temperature was recorded initially and then at five minute intervals.

An initial study was performed to develop the animal model. In this part of the procedure 14 animals were placed in the ice boxes and removed once core temperatures dropped below 26 °C. They were then placed on a bench top to be stabilized with polar fleece blanket "sleeping bags" that had been specially designed for the procedure (Fig. 2). The sleeping bags enabled the core temperatures to stabilize at around 25–26 °C for the remainder of the procedure. The anaesthetized rats were euthanized by cervical dislocation after 120 min. Having established the model, the second part of the procedure consisted of lowering the core temperatures to ~10 °C in the remaining 28 rats, resulting in death of the animals under anaesthesia at variable times from 120 to 240 min. The animals were then subjected to necropsy with immediate, and later microscopic, examination of the gastric mucosa. This study was fully approved by the Animal Ethics Committee at The University of Adelaide.

Fig. 1. Two anaesthetized rats placed on a drape cloth covered mesh platform in a styrofoam box packed with ice. The animals were surrounded by, and in contact with, ice but were not covered, to enable visual monitoring of their clinical status.

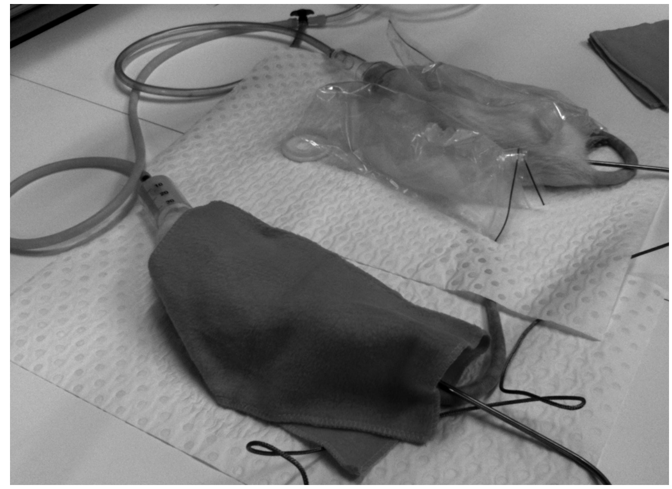

Fig. 2. In the initial part of the study to develop the model 14 rats were removed from the ice boxes once their core body temperatures had dropped to 26 °C and were then stabilized in polar fleece sleeping bags at around 25–26 °C.

3. Results

The rate of fall of rectal core temperatures in the 42 rats is shown in Fig. 3. There were no significant differences in the decline in core temperatures between the two age groups (young vs old) although the younger animals tended to survive for a longer time. Macroscopic examination of the gastric mucosa in all animals revealed no discernible abnormalities (Fig. 4). Specifically there was no evidence of Wischnewski spots in the gastric mucosa from the 14 rats that were stabilized under anaesthesia and euthanised at 120 min. Similarly there were no abnormalities observed on macro- and microscopic examination of the gastric mucosa from the

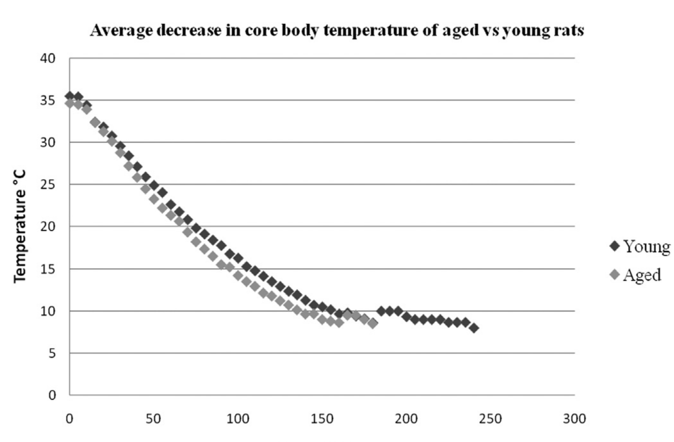

Fig. 3. Decrease in average core body temperature over time of 21 young vs 21 aged male Sprague–Dawley rats exposed to cold for periods of 2–4 h.

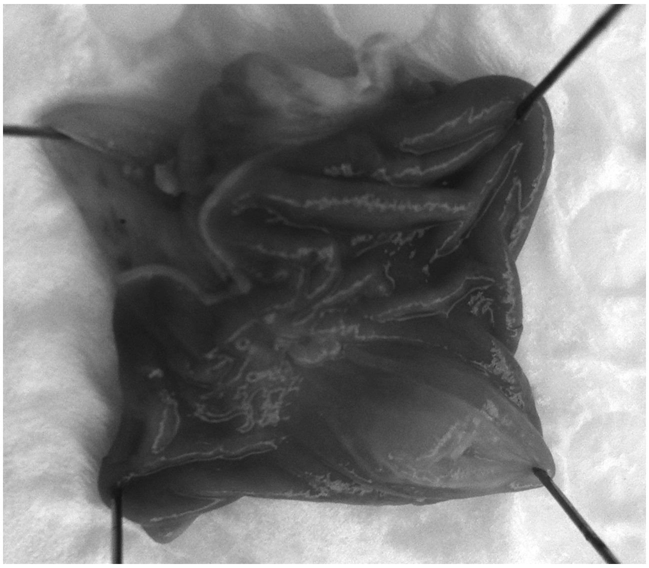

Fig. 4. Excised stomach opened to show the gastric mucosa of a rat exposed to hypothermic conditions for 4 h, with core body temperatures reduced to below 10 °C. No macroscopic changes are evident.

remaining 28 rats that died of hypothermia under anaesthesia between 120 and 240 min.

4. Discussion

As noted above, hypothermia may be a difficult diagnosis to make post mortem. Scene features such as low environmental temperatures, incapacitation on cold floors, paradoxical undressing or "hide and die" behaviour may be significant clues,[1,2] as may conditions such as diabetes mellitus.[18] However pathological findings that may be relied upon for diagnosis, such as Wischnewski spots (Fig. 5), may vary considerably in their incidence between jurisdictions.[1] Whether this depends on local diagnostic practices or on the inter-action of a variety of complex factors remains unclear.

Although an age related vulnerability to the development of Wischnewski spots has been reperted,[12] this was not observed in the current study. The vulnerability to the effects of hypothermia with increasing age[17] was, however, confirmed (Fig. 3).

Previously in the literature, a number of studies have reported the successful generation of Wischnewski spots in rodent models associated with both stress and low temperatures. The technique described in the current study represents a consistent and reproducible method for stabilizing rats taken from a relatively low stress environment at low temperatures for a number of hours so that the pathological consequences of hypothermia in isolation can be evaluated. The absence of Wischnewski spots in animals that had died of hypothermia after a number of hours of being exposed to the cold provides further support for stress being an import cofactor in the development of these lesions.

Under-diagnosis of lethal hypothermia is an ever present problem in many forensic practices. This may occur in warmer climates particularly among elderly, socially-isolated individuals who live in poorly heated/insulated dwellings.[6,19] It may also arise due to the lack of sensitivity of a number of pathological markers such as Wischnewski spots. Future use of the animal model developed for this study may enable greater understanding of the role of lowered body temperature alone as an initiator of the pathological changes noted at autopsy. It may also help to clarify the contribution of stress to the pathogenesis of markers that are relied upon so heavily by pathologists for the diagnosis.

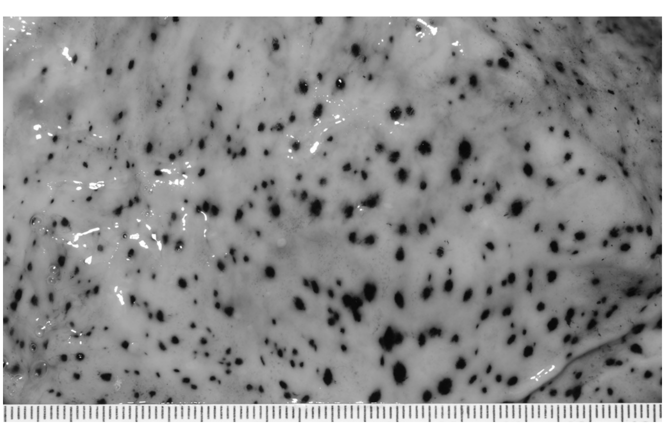

Fig. 5. Wischnewski spots in the gastric mucosa of a human exposed to significant hypothermia to demonstrate the typical appearance of these lesions.

Ethical approval

The University of Adelaide Animal Ethics Committee.

Funding

None.

Conflict of interest

None.

References

1. Bright F, Winskog C, Byard RW. Wischnewski ulcers and hypothermia – sensitive, specific or serendipitous? *Forensic Sci Med Pathol* 2013;**9**:88–90.
2. Turk EE. Hypothermia. *Forensic Sci Med Pathol* 2010;**6**:106–15.
3. Madea B, Tsokos M, Preub J. Morphological findings, their pathogenesis and diagnostic value. *Forensic Pathol Rev* 2008;**5**:3–21.
4. Dettmeyer RB. Hypothermia. *Forensic Histopathol* 2011;**8**:165–71.
5. Zhou C, Byard RW. Armanni-Ebstein phenomenon and hypothermia. *Forensic Sci Int* 2011;**206**:e82–4.
6. Bright F, Winskog C, Gilbert JD, Byard RW. Additional risk factors for lethal hypothermia. *J Forensic Leg Med* 2013. in press.
7. Birchmeyer MS, Mitchell EK. Wischnewski revisited: the diagnostic value of gastric mucosa ulcers in hypothermic deaths. *Am J Forensic Med Pathol* 1989;**10**:28–30.
8. Tsokos M, Rothschild M, Madea B, Risse M, Sperhake JP. Histological and immunohostochemical study of Wischnewsky spots in fatal hypothermia. *Am J Forensic Med Pathol* 2006;**27**:70–4.
9. Wolf DA, Aronson J, Rajaraman S, Veasey SP. Wischnewski ulcers and acute pancreatitis in two hospitalized patients with cirrhosis, portal vein thrombosis and hypothermia. *J Forensic Sci* 1999;**44**:1082–5.
10. Hirvonen J, Elfving R. Histamine and serotonin in the gastric erosions of rats dead from exposure to cold: a histochemical and quantitative study Zeitschrift für Rechtsmedizin. *Int J Leg Med* 1974;**74**:273–81.
11. Sperhake JP, Rothschild M, Risse M, Tsokos M. Histomorphology of Wischnewsky spots: contribution to the forensic histopatology of fatal hypothermia. In: Oehmichen M, editor. *Hypothermia. Clinical, pathomorphological and forensic features*. Lubek: Schmidt-Romhild; 2004. p. 211–20.
12. Takada M, Kusano I, Yamamoto H, Shiraishi T, Yatani R, Haba K. Wischnewsky's gastric lesions in accidental hypothermia. *Am J Forensic Med Pathol* 1991;**12**:300–5.
13. Landeira-Fernandez J. Analysis of the cold-water restraint procedure in gastric ulceration and body temperature. *Physiol Behav* 2004;**82**:827–33.
14. Vincent GP, Pare WP, Prenatt JED. Aggression, body temperature and stress ulcer. *Physiol Behav* 1984;**32**:265–8.
15. Goldman H, Rosoff CB. Pathogenesis of acute gastric stress ulcers. *Am J Pathol* 1968;**52**:227–44.
16. Wagner JA, Horvath S. Influence of age and gender on human thermoregulatory responses to cold exposures. *J Am Physiolog Soc* 1985;**85**:180–6.
17. Kiang-Ulrich M, Horvath S. Age-related differences in response to acute cold challenge (−10 °C) in male F344 rats. *Exp Gerontol* 1985;**20**:201–2.
18. Byard RW, Zhou C. Erosive gastritis, Armanni-Ebstein phenomenon and diabetic ketoacidosis. *Forensic Sci Med Pathol* 2010;**6**:304–6.
19. Bright F, Winskog C, Walker M, Byard RW. An analysis of hypothermic deaths in South Australia compared to Sweden. *J Forensic Sci* (in press).